



**LIBBY ASBESTOS SUPERFUND SITE
OPERABLE UNIT 3
SURFACE WATER TOXICITY TESTING PROTOCOL**

1.0 INTRODUCTION

Surface water in the Rainy Creek drainage contains Libby Amphibole asbestos (LA). The toxicity of this material to fish is not known. This document describes the basic protocol that will be followed to evaluate the toxicity of site waters to fish.

The protocol presented here is based in part on the protocol developed by Belanger (1985).

2.0 PROTOCOL

Test Waters

All test waters will be stored in the dark at 1-4°C prior to use in the tests. This is to minimize the growth of algae or any other biological organisms.

Reference Water

The reference water will be moderately hard reconstituted laboratory water, prepared in accord with Section 7.2.3 of EPA (2002).

Site Waters

Test waters will be provided to the toxicity testing laboratory by EPA. Initial studies will utilize waters collected from one or more onsite locations. Subsequent tests may include preparing a dilution series of an on-site water, or use of laboratory water “spiked” with LA.

Comment [DW1]: Recommend deleting. EPA won't be providing the water correct?

Water Chemistry

All site waters will be characterized by EPA by analysis for LA, dissolved and total metals, pH, and hardness. All test waters (including reference water) will be monitored in the laboratory for the following parameters:

Parameter	Frequency
Temperature	Daily
pH	Daily
Dissolved oxygen	once per 5 days before swimup, and then at the start and end of each static renewal
Ammonia	

Test Species

The test species will be rainbow trout (*Oncorhynchus mykiss*)

Life Stage

The life stage will be newly hatched larvae.

Exposure Conditions

Exposure will be performed using a static renewal protocol in 4-Liter aquaria. There will be 15 larvae per aquarium, with three aquaria per test water (a total of 45 larvae per test water).

Water temperature will be maintained at $12 \pm 1^{\circ}\text{C}$.

Exposure duration will be 6 weeks (42 days).

During the larval stage, water will be changed once every 10 days.

Swim-up is expected to occur on or about day 20 (after about 240 degree-days). After swim-up occurs, water will be changed once every three days. Based on this design, the minimum total volume of water required for each test is approximately 120 L.

In order to ensure that fiber settling does not occur, each aquarium will be equipped with an air bubbler placed in the bottom and run continuously. In addition, each aquarium will be equipped with a circulating filter, used without any filter media.

Feeding

No feeding will occur during the larval stage.

After swim-up occurs, fish will be fed freshly-hatched brine shrimp (about 12 hours post hatch) daily at a rate of 0.05 grams of brine shrimp per gram of fish in the aquariums. The average mass of each fish as a function of time may be estimated from measurements on fish grown in parallel tanks, or from historical growth curves.

Endpoints

Behavior

All aquaria will be observed daily for indications of differences in behavior between control fish and fish exposed to site waters. This may include, for example, differences in the frequency and duration of swimming events of the larvae, swimming and feeding behavior of the fry, etc. These observations will be recorded using the behavioral observation log sheet provided as Attachment 1.

Mortality

Observations on mortality will be recorded twice daily at approximately 8:30 AM and 4:30 PM. Attachment 2 is a form that will be used for assigning a unique identifies to each fish and for recording date and time of death of each fish.

Histopathology

All fish that die during the study and all fish alive at the end of the study will be preserved by being placed into fixative solution for subsequent [quantitative](#) histopathological evaluation. Detailed SOPs for sample preservation, slide preparation, and histological examination will be provided by the histological laboratory.

Growth

No measures of growth will be performed during the larval stage. After swim-up, measures of growth will include length and mass of the fish. Data on growth will be recorded using the form provided as Attachment 2

REFERENCES

Belanger, S. E. 1985. Functional and pathological Responses of Selected Aquatic Organisms to Chrysotile Asbestos. Doctoral Dissertation approved by Virginia Polytechnic Institute and State University, September 1985.

EPA. 2002. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. U.S. Environmental Protection Agency. EPA-821-R-02-013. October, 2002.

ATTACHMENT 1. BEHAVIORAL LOG

Water Sample = _____ Study Start Date = _____

Day	Observations
1	
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ATTACHMENT 2. MORTALITY AND GROWTH LOG

Test water description =
 Test water code =

Mill Pond
 MP

Aquarium	Fish	Label Assigned	Day of death	Time death was noted	Length (mm)	Weight (mg)	Notes
A	1	MP-A1					
	2	MP-A2					
	3	MP-A3					
	4	MP-A4					
	5	MP-A5					
	6	MP-A6					
	7	MP-A7					
	8	MP-A8					
	9	MP-A9					
	10	MP-A10					
	11	MP-A11					
	12	MP-A12					
	13	MP-A13					
	14	MP-A14					
	15	MP-A15					
B	1	MP-B1					
	2	MP-B2					
	3	MP-B3					
	4	MP-B4					
	5	MP-B5					
	6	MP-B6					
	7	MP-B7					
	8	MP-B8					
	9	MP-B9					
	10	MP-B10					
	11	MP-B11					
	12	MP-B12					
	13	MP-B13					
	14	MP-B14					
	15	MP-B15					
C	1	MP-C1					
	2	MP-C2					
	3	MP-C3					
	4	MP-C4					
	5	MP-C5					
	6	MP-C6					
	7	MP-C7					
	8	MP-C8					
	9	MP-C9					
	10	MP-C10					
	11	MP-C11					
	12	MP-C12					
	13	MP-C13					
	14	MP-C14					
	15	MP-C15					